

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (Previously presented) A method of amplifying a template DNA molecule comprising:
incubating said template DNA molecule with a reaction mixture comprising a DNA polymerase and at least one accessory protein at a constant temperature to produce amplified product, wherein production of amplified product does not require exogenously-added oligonucleotide primers and said template DNA molecule does not have a terminal protein covalently bound to either 5' end, and wherein said method is performed under conditions such that the amount of amplified product is at least 10-fold greater than the amount of template DNA put into the mixture.

2 – 10. (Canceled)

11. (Previously presented) A method of amplifying a template DNA molecule comprising:
incubating said template DNA molecule with an *in vitro* reaction mixture comprising a DNA polymerase, a helicase, and a primase at a constant temperature to produce amplified product, wherein said method is performed under conditions such that the amount of amplified product is at least 10-fold greater than the amount of template DNA put into the mixture.

12 – 23. (Canceled)

24. (Currently amended) A method of amplifying a template DNA molecule comprising:

incubating said template DNA molecule in an *in vitro* reaction mixture comprising a wild-type T7 DNA polymerase and a T7 DNA polymerase modified to have reduced 3' to 5' exonuclease activity, a 63-kDa form of a gene 4 protein from bacteriophage T7 and a single-stranded binding protein from *Escherichia coli* at a constant temperature to produce amplified product, wherein production of amplified product does not require exogenously-added

oligonucleotide primers **and the amount of amplified product is at least 10-fold greater than the amount of template DNA put into the mixture.**

25 – 123. (Canceled)

124. (Previously presented) The method of claim 1, wherein said method is performed under conditions such that the amount of amplified product is at least 100-fold greater than the amount of template DNA put into the mixture.

125. (Previously presented) The method of claim 1, wherein said method is performed under conditions such that the amount of amplified product is at least 1,000-fold greater than the amount of template DNA put into the mixture.

126. (Previously presented) The method of claim 1, wherein said method is performed under conditions such that the amount of amplified product is at least 1,000,000-fold greater than the amount of template DNA put into the mixture.

127. (Previously presented) The method of claim 1, wherein said method is performed under conditions such that the amount of amplified product is at least 10,000,000-fold greater than the amount of template DNA put into the mixture.

128. (Previously presented) The method of claim 1, wherein said method is performed under conditions such that amplification of template DNA is exponential.

129. (Previously presented) The method of claim 1, wherein said DNA polymerase comprises a combination of two forms of T7 DNA polymerase.

130. (Previously presented) The method of claim 1, wherein said DNA polymerase is a bacteriophage DNA polymerase.

131. (Previously presented) The method of claim 1, wherein said DNA polymerase is a bacteriophage T7 DNA polymerase.

132. (Previously presented) The method of claim 1, wherein said DNA polymerase comprises a mixture of a T7 DNA polymerase with a normal level of exonuclease activity and a T7 DNA polymerase modified to have reduced 3' to 5' exonuclease activity.

133. (Previously presented) The method of claim 132, wherein said T7 DNA polymerase with a normal level of exonuclease activity has about 5,000 units of exonuclease activity per mg protein.

134. (Previously presented) The method of claim 132, wherein said T7 DNA polymerase modified to have reduced 3' to 5' exonuclease activity has less than 50% of the 3' to 5' exonuclease activity of said T7 DNA polymerase with a normal level of exonuclease activity.

135. (Previously presented) The method of claim 132, wherein the molar ratio of said T7 DNA polymerase modified to have reduced 3' to 5' exonuclease activity to said T7 DNA polymerase with a normal level of exonuclease activity is greater than 1.

136. (Previously presented) The method of claim 132, wherein the molar ratio of said T7 DNA polymerase modified to have reduced 3' to 5' exonuclease activity to said T7 DNA polymerase with a normal level of exonuclease activity is approximately 20:1.

137. (Previously presented) The method of claim 1, wherein said accessory protein is a helicase.

138. (Previously presented) The method of claim 1, wherein said accessory protein is a primase.

139. (Previously presented) The method of claim 1, wherein said accessory protein is the helicase/primase from bacteriophage T7.

140. (Previously presented) The method of claim 139, wherein said helicase/primase is the 63-kDa form of the protein from bacteriophage T7.

141. (Previously presented) The method of claim 1, wherein said reaction mixture further comprises a single-stranded DNA binding protein.

142. (Previously presented) The method of claim 141, wherein said single-stranded DNA binding protein is from *Escherichia coli*.

143. (Currently amended) The method of claim 1, wherein said constant temperature is less than **about** 60° C.

144. (Currently amended) The method of claim 1, wherein said constant temperature is less than **about** 50° C.

145. (Currently amended) The method of claim 1, wherein said constant temperature is less than **about** 45° C.

146. (Currently amended) The method of claim 1, wherein said constant temperature is less than **about** 40° C.

147. (Previously presented) The method of claim 1, wherein said constant temperature is about 37° C.

148. (Previously presented) The method of claim 1, wherein the reaction mixture further comprises one or more reagents selected from the group consisting of a nucleoside diphosphokinase, an inorganic pyrophosphatase, an ATP regeneration system and a ligase.

149. (Previously presented) The method of claim 1, wherein the reaction mixture further comprises a nucleoside diphosphokinase.

150. (Previously presented) The method of claim 1, wherein the reaction mixture further comprises an inorganic pyrophosphatase.

151. (Previously presented) The method of claim 1, wherein the reaction mixture further comprises an ATP regeneration system.

152. (Previously presented) The method of claim 151, wherein said ATP regeneration system comprises a combination of creatine kinase and phosphocreatine.

153. (Previously presented) The method of claim 1, wherein the reaction mixture further comprises a ligase.

154. (Previously presented) The method of claim 153, wherein said ligase is bacteriophage T7 DNA ligase.

155. (Previously presented) The method of claim 1, wherein the reaction mixture further comprises one or more additives selected from the group consisting of potassium glutamate, DMSO and dextran polymer.

156. (Previously presented) The method of claim 11, wherein said method is performed under conditions such that production of amplified product does not require exogenously-added oligonucleotide primers.

157. (Previously presented) The method of claim 11, wherein said method is performed under conditions such that the amount of amplified product is at least 100-fold greater than the amount of template DNA put into the mixture.

158. (Previously presented) The method of claim 11, wherein said method is performed under conditions such that the amount of amplified product is at least 1,000,000-fold greater than the amount of template DNA put into the mixture.

159. (Previously presented) The method of claim 11, wherein said method is performed under conditions such that amplification of template DNA is exponential.

160. (Cancelled).

161. (Previously presented) The method of claim 24, wherein said method is performed under conditions such that the amount of amplified product is at least 100-fold greater than the amount of template DNA put into the mixture.

162. (Previously presented) The method of claim 24, wherein said method is performed under conditions such that the amount of amplified product is at least 1,000-fold greater than the amount of template DNA put into the mixture.

163. (Previously presented) The method of claim 24, wherein said method is performed under conditions such that the amount of amplified product is at least 1,000,000-fold greater than the amount of template DNA put into the mixture.

164. (Previously presented) The method of claim 24, wherein said method is performed under conditions such that amplification of template DNA is exponential.

165. (Previously presented) A method of amplifying a template DNA molecule comprising:

incubating said template DNA molecule with a reaction mixture comprising a DNA polymerase and at least one accessory protein at a constant temperature to produce amplified product, wherein production of amplified product does not require exogenously-added oligonucleotide primers and said template DNA molecule does not have a terminal protein covalently bound to either 5' end, and

wherein said DNA polymerase comprises a mixture of a T7 DNA polymerase with a normal level of exonuclease activity and a T7 DNA polymerase modified to have reduced 3' to 5' exonuclease activity.

166. (Previously presented) A method of amplifying a template DNA molecule comprising:

incubating said template DNA molecule with a reaction mixture comprising a DNA polymerase and at least one accessory protein at a constant temperature to produce amplified product, wherein production of amplified product does not require exogenously-added

oligonucleotide primers and said template DNA molecule does not have a terminal protein covalently bound to either 5' end, and

wherein said method is performed under conditions such that amplification of template DNA is exponential.

167. (Canceled).

168. (Previously presented) A method of amplifying a template DNA molecule comprising: incubating said template DNA molecule with an *in vitro* reaction mixture comprising a DNA polymerase, a helicase, and a primase at a constant temperature to produce amplified product,

wherein said method is performed under conditions such that amplification of template DNA is exponential.

169. (Previously presented) A method of amplifying a template DNA molecule comprising: incubating said template DNA molecule in an *in vitro* reaction mixture comprising a wild-type T7 DNA polymerase and a T7 DNA polymerase modified to have reduced 3' to 5' exonuclease activity, a 63-kDa form of a gene 4 protein from bacteriophage T7 and a single-stranded binding protein from *Escherichia coli* at a constant temperature to produce amplified product,

wherein said method is performed under conditions such that amplification of template DNA is exponential.